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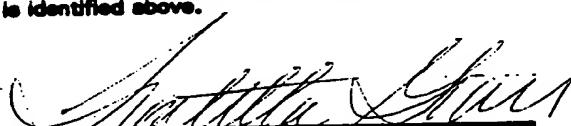
BAR CODE LABEL 		U.S. PATENT APPLICATION			
SERIAL NUMBER 08/582,825		FILING DATE 01/04/96	CLASS 435	GROUP ART UNIT 1816	
APPLICANT MING-SHI CHANG, NEWBURY PARK, CA.	REC'D 26 FEB 1997 PCT				
**CONTINUING DATA***** VERIFIED					
**FOREIGN/PCT APPLICATIONS***** VERIFIED					
<b>PRIORITY DOCUMENT</b>					
FOREIGN FILING LICENSE GRANTED 05/17/96					
STATE OR COUNTRY CA	SHEETS DRAWING 0	TOTAL CLAIMS 19	INDEPENDENT CLAIMS 6	FILING FEE RECEIVED \$1,364.00	ATTORNEY DOCKET NO. A-382
ADDRESS US PATENT OPERATIONS IMP MS 10 1 B AMGEN INC AMGEN CENTER 1840 DEHAVILLAND DRIVE THOUSAND OAKS CA 91320-1789					
TITLE OF PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS					
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EXHIBIT 3  
Nika Adham, et al.  
Serial No.: 09/116,676  
Filed: July 16, 1998

#12 Attachment  
09/116676

PATENT APPLICATION SERIAL NO. 0/582825

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE  
Fee Record Sheet

300 01-0519 02/01/96 08582825  
30077 101 1,234.00CH



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PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

5 The present invention relates to OB protein receptors, related compositions and methods of making and using such receptor and related compositions.

BACKGROUND

10 Although the molecular basis for obesity is largely unknown, the identification of the "OB gene" and protein encoded ("OB protein") has shed some light on mechanisms the body uses to regulate body fat deposition. Zhang et al., Nature 372: 425-432 (1994); see also, the Correction at Nature 374: 479 (1995). The OB protein is active *in vivo* in  
15 both ob/ob mutant mice (mice obese due to a defect in the production of the OB gene product) as well as in normal, wild type mice. The biological activity manifests itself in, among other things, weight loss. See generally, Barinaga, "Obese" Protein Slims Mice, Science 269: 475-476 (1995).

20 The other biological effects of OB protein are not well characterized. It is known, for instance, that in ob/ob mutant mice, administration of OB protein results in a decrease in serum insulin levels, and serum glucose levels. It is also known that administration of OB protein results in  
25 a decrease in body fat. This was observed in both ob/ob mutant mice, as well as non-obese normal mice. Pelleymounter et al., Science 269: 540-543 (1995); Halaas et al., Science 269: 543-546 (1995). See also, Campfield et al., Science 269: 546-549 (1995) (Peripheral and central administration of  
30 microgram doses of OB protein reduced food intake and body weight of ob/ob and diet-induced obese mice but not in db/db obese mice.) In none of these reports have toxicities been observed, even at the highest doses.

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Date of Deposit January 4, 1996

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Despite the promise of clinical application of the OB protein, the mode of action of the OB protein *in vivo* is not clearly elucidated, in part due to the absence of information on the OB receptor. High affinity binding of the 5 OB protein has been detected in the rat hypothalamus, reportedly indicating OB receptor location. Stephens et al., Nature 377: 530-532. The *db/db* mouse displays the identical phenotype as the *ob/ob* mouse, i.e., extreme obesity and Type 10 II diabetes; this phenotype is thought to be due to a defective OB receptor, particularly since *db/db* mice fail to respond to OB protein administration. See Stephens et al., *supra*.

Identification of the OB protein receptor is key in determining the pathway of signal transduction. Moreover, 15 identification of the OB protein receptor would provide powerful application in diagnostic uses, for example, to determine if individuals would benefit from OB protein therapy. Furthermore, the OB receptor could be a key component in an assay for determining additional molecules 20 which bind to the receptor and result in desired biological activity.

SUMMARY OF THE INVENTION

The present invention relates to a novel class of 25 protein receptors, herein denominated "OB protein receptors" or "OB receptors", which are thought to selectively bind OB protein. As such, the novel OB receptor family is provided, as well as novel members of such family. Also provided are nucleic acids, vectors and host cells containing such nucleic 30 acids, related antisense nucleic acids, molecules which selectively bind to the OB protein receptor, and related compositions of matter. In other aspects, the present invention relates to methods of using the above compositions, such as diagnostic methods, and methods for preparing OB 35 receptor ligands.

DETAILED DESCRIPTION

A novel family of OB receptors is provided. This novel family resulted from identification of a PCR fragment isolated from a human liver cell cDNA library. The original PCR fragment, from which primers were isolated, contained a "WSXWS" motif, common to cytokine receptors. As illustrated by the working examples below, using this fragment three members of this OB protein receptor family have been identified. These members, herein designated as "A", "B", and "C", are identical at amino acid position 1-891 (using the numbering of Seq. ID No. 1), but diverge at position 892 through the C terminus. They vary in length at the C-terminus beyond amino acid 891, and the different forms appear to have different tissue distribution.

Ligand binding may be localized to the extracellular domain of the OB receptor. Using hydrophobicity analysis, the leader sequence is likely to comprise amino acids (Seq. ID. No. 1) 1-21, 1-22, or 1-28. The first amino acid of the mature protein is likely to be 22(F), 23(N) or 29(T). Most likely, the first amino acid of the mature protein is 22 (F). The beginning of the transmembrane domain appears to be located at position 839(A) or 841(L). The end of the transmembrane domain appears to be located at position 862(I), 863(S) or 864(H). Thus, based on predictions from hydrophobicity analysis, for OB protein binding, at a minimum what is needed is the extracellular domain of the mature protein, amino acids 22, 23 or 29 through amino acids 839(D) or 841(G). Therefore, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):  
(a) 1-896;  
(b) 22-896;  
(c) 23-896;  
(d) 29-896

- (e) 1-839;
- (f) 22-839;
- (g) 29-839;
- (h) 1-841;
- 5 (i) 22-841;
- (j) 23-841;
- (k) 29-841;
- (l) 1-891;
- (m) 22-891;
- 10 (n) 23-891;
- (o) 29-891;
- (p) the amino acids of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);
- 15 (q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

The C-terminus region is intracellular. The differences in the C-terminus among members of the present OB receptor family may result in differences in signal transduction among the species. Thus, the present OB receptors include at least the extracellular domain which is important for OB protein ligand binding. Nucleic acids encoding the present OB receptors, vectors, and host cells are also provided for herein.

Human genomic DNA is also provided herein. The genomic DNA has been localized to human chromosome 1P31, which is believed to correspond to mouse chromosome 4, the presumed location of the mouse db locus.

30 Tissue distribution analysis demonstrates the presence of OB receptor nucleic acids is fairly ubiquitous, and particularly noted in the liver. It is also observed in the ovary, and heart; and, to a lesser extent, in small intestine, lung, skeletal muscle, kidney, and, to an even

lesser extent, spleen, thymus, prostate, testes, Placenta and pancreas.

Nucleic Acids

- 5 According to the present invention, novel OB protein receptors and DNA sequences coding for all or part of such OB receptors are provided. Novel nucleic acid sequences of the invention include sequences useful in securing expression in prokaryotic or eucaryotic host cells of
- 10 polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of recombinant human OB receptor. The nucleic acids may be purified and isolated, so that the desired coding region is useful to produce the present polypeptides,
- 15 for example, or for diagnostic purposes, as described more fully below. DNA sequences of the invention specifically comprise: (a) any of the DNA sequence set forth in Seq. ID No. 2, 4, 6 and 7 (and complementary strands); (b) a DNA sequence which hybridizes (under hybridization conditions
- 20 disclosed in the cDNA library screening section below, or equivalent conditions or more stringent conditions) to the DNA sequence in subpart (a) or to fragments thereof; and (c) a DNA sequence which, but for the degeneracy of the genetic code, would hybridize to the DNA sequence in subpart (a).
- 25 Specifically comprehended in parts (b) and (c) are genomic DNA sequences encoding allelic variant forms of human OB receptor and/or encoding OB receptor from other mammalian species, and manufactured DNA sequences encoding OB receptor, fragments of OB receptor, and analogs of OB receptor which
- 30 DNA sequences may incorporate codons facilitating transcription and translation of messenger RNA in microbial hosts. Such manufactured sequences may readily be constructed according to the methods of Alton et al., PCT published application WO 83/04053.

Genomic DNA encoding the present OB receptors may contain additional non-coding bases, or introns, and such genomic DNAs are obtainable by hybridizing all or part of the cDNA, illustrated in Seq. ID NOS. 1, 3, and 5, to a genomic DNA source, such as a human genomic DNA library. Such genomic DNA will encode functional OB receptor polypeptide; however, use of the cDNAs may be more practicable in that, since only the coding region is involved, recombinant manipulation is facilitated.

Such sequences include the incorporation of codons "preferred" for expression by selected nonmammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences which facilitate construction of readily expressed vectors. The present invention also provides DNA sequences coding for polypeptide analogs or derivatives of OB receptor which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (i.e., deletion analogs containing less than all of the residues specified for OB receptor; substitution analogs, wherein one or more residues specified are replaced by other residues; and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptide) and which share some or all the biological properties of OB receptor.

Such substitutions may include the substitution of serine or alanine at one or more of the cysteinyl residues, conserved amino acid substitutions (conserved in terms of charge, hydrophobicity or both) or other substitutions. The leader sequence DNA may be substituted with another leader sequence for ease in expression or for other purposes.

The present DNA sequences may be selected from among those encoding OB receptors including those having amino acids (according to Seq. ID No. 1):

- (a) 1-896;
  - (b) 22-896;
  - (c) 23-896;
  - (d) 29-896
- 5        (e) 1-839;
- (f) 22-839;
  - (g) 29-839;
  - (h) 1-841;
  - (i) 22-841;
- 10      (j) 23-841;
- (k) 29-841;
  - (l) 1-891;
  - (m) 22-891;
  - (n) 23-891;
- 15      (o) 29-891;
- (p) the amino acids of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5); and
  - (q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o,
- 20      and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

The present invention also includes human genomic DNA.

- In addition, since the C-terminus region of the
- 25 above polyptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3, and 5) one may desire to prepare only that portion of the polypeptides which are divergent. As such, DNA sequences are provided which encode polypeptides:
- (a) having only amino acids 892-896 of Seq. ID No. 1;
  - 30 (b) having only amino acids 892-933 of Seq. ID No. 3;
  - (c) having only amino acids 892-959 of Seq. ID No. 5.

Also, one may prepare antisense nucleic acids against the present DNAs. Such antisense nucleic acids may be useful in modulating the effects of OB protein *in vivo*.

- 35 For example, one may prepare an antisense nucleic acid which

effectively disables the ability of a cell to produce OB receptor by binding to the nucleic acid which encodes such OB receptor.

DNA sequences of the invention are also suitable  
5 materials for use as labeled probes in isolating human  
genomic DNA encoding OB receptor, as mentioned above, and  
related proteins as well as cDNA and genomic DNA sequences of  
other mammalian species. DNA sequences may also be useful in  
various alternative methods of protein synthesis (e.g., in  
10 insect cells) or, as described above, in genetic therapy in  
humans and other mammals. DNA sequences of the invention are  
expected to be useful in developing transgenic mammalian  
species which may serve as eucaryotic "hosts" for production  
of OB receptor and OB receptor products in quantity. See,  
15 generally, Palmiter et al., Science 222: 809-814 (1983).

Vectors and Host Cells

According to another aspect of the present  
invention, the DNA sequences described herein which encode OB  
20 receptor polypeptides are valuable for the information which  
they provide concerning the amino acid sequence of the  
mammalian protein which have heretofore been unavailable.  
Put another way, DNA sequences provided by the invention are  
useful in generating new and useful viral and circular  
25 plasmid DNA vectors, new and useful transformed and  
transfected procaryotic and eucaryotic host cells (including  
bacterial and yeast cells and mammalian cells grown in  
culture), and new and useful methods for cultured growth of  
such host cells capable of expression of OB receptor and its  
30 related products.

The DNA provided herein (or corresponding RNAs) may  
also be used for gene therapy for, example, treatment of  
conditions characterized by the overexpression of OB protein,  
such as anorexia or cachexia. Alternatively, gene therapy may  
35 be used in cases where increased sensitivity to OB protein is

desired, such as in cases where an individual has a condition characterized by OB protein receptors defective in ability to bind or retain the binding of OB protein. Currently, vectors suitable for gene therapy (such as retroviral or adenoviral 5 vectors modified for gene therapy purposes and of purity and pharmaceutical acceptability) may be administered for delivery into the lung, for example. Such vectors may incorporate nucleic acid encoding the present polypeptides for expression in a desired location. Gene therapy may 10 involve a vector containing more than one gene for a desired protein.

Alternatively, one may use no vector so as to facilitate relatively stable presence in the host. For example, homologous recombination may facilitate integration 15 into a host genome. (This may be performed for production purposes as well, e.g., U.S. Patent No. 5,272,071 and WO 91/09955.) The nucleic acid may be placed within a pharmaceutically acceptable carrier to facilitate cellular uptake, such as a lipid solution carrier (e.g., a charged 20 lipid), a liposome, or polypeptide carrier (e.g., polylysine). A review article on gene therapy is Verma, Scientific American, November 1990, pages 68-84 which is herein incorporated by reference.

As mentioned above, target cells may be within the 25 lungs of the recipient, but other target cells may be adipocytes or precursors thereof, bone marrow cells, blood cells, such as peripheral blood progenitor cells, liver (or other organ) cells, muscle cells, fibroblasts, or other cells. The desired nucleic acid may be first placed within a 30 cell, and the cell may be administered to a patient (such as a transplanted tissue) or the desired nucleic acid may be administered directly to the patient for uptake *in vivo*.

The cells to be transferred to the recipient may be cultured using one or more factors affecting the growth or

proliferation of such cells, as for example, SCF if appropriate..

For gene therapy dosages, one will generally use between one copy and several thousand copies of the present 5 nucleic acid per cell, depending on the vector, the expression system, the age, weight and condition of the recipient and other factors which will be apparent to those skilled in the art.

Thus, the present invention provides for a 10 population of cells expressing an OB receptor of the present OB receptor family. Such cells are suitable for transplantation or implantation into an individual for therapeutic purposes. For example, one may prepare a population of cells to overexpress OB receptor (such as one 15 identified in the Sequence ID's or otherwise denoted herein), or to express a desired form of OB receptor, such as one which is particularly sensitive to OB protein. One may then implant such cells into an individual to increase that individual's sensitivity to OB protein. Such cells may, for 20 example, be liver cells, or bone marrow cells. Alternatively, one may wish to use overexpressing circulating cells such as blood progenitor cells or other blood cells. Cells may be in the form of tissue. Such cells may be cultured prior to transplantation or implantation. Such OB receptor 25 overexpression, or expression of particularly sensitive forms of OB receptor may be accomplished by, for example, altering the regulatory mechanism for expression of OB receptor, such as using homologous recombination techniques as described supra. Thus, provided is a population of host cells modified 30 so that expression of endogenous OB receptor DNA is enhanced.

The present invention provides purified and isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of 35 amino acid residues) and one or more of the biological

properties (e.g., immunological properties and in vitro biological activity) and physical properties (e.g., molecular weight) of naturally-occurring mammalian OB receptor including allelic variants thereof. The term "purified and isolated" herein means substantially free of unwanted substances so that the present polypeptides are useful for an intended purpose. For example, one may have a recombinant human OB receptor substantially free of other human proteins or pathological agents. These polypeptides are also characterized by being the a product of mammalian cells, or the product of chemical synthetic procedures or of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. The products of expression in typical yeast (e.g., Saccharomyces cerevisiae) or procaryote (e.g., E. coli) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g., non-human mammalian (e.g. COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, and other factors, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be non-glycosylated. One may modify the nucleic acid so that glycosylation sites are included. Polypeptides of the invention may also include an initial methionine amino acid residue (at position -1 with respect to the first amino acid residue of the mature polypeptide).

In addition to naturally-occurring allelic forms of OB receptor, the present invention also embraces other OB receptor products such as polypeptide analogs of OB receptor and fragments of OB receptor. Following the procedures of the above noted published application by Alton et al. (WO 83/04053), one can readily design and manufacture genes coding for microbial expression of polypeptides having

primary conformations which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternately, modifications of cDNA and genomic genes may be readily accomplished by well-known site-directed mutagenesis techniques and employed to generate analogs and derivatives of OB receptor. Such products would share at least one of the biological properties of mammalian OB receptor but may differ in others. As examples, projected 5 products of the invention include those which are foreshortened by e.g., deletions; or those which are more stable to hydrolysis (and, therefore, may have more pronounced or longer lasting effects than naturally-occurring); or which have been altered to delete one or more 10 potential sites for glycosylation (which may result in higher activities for yeast-produced products); or which have one or more cysteine residues deleted or replaced by, e.g., alanine or serine residues and are potentially more easily isolated 15 in active form from microbial systems; or which have one or more tyrosine residues replaced by phenylalanine and bind 20 more or less readily to target proteins or to receptors on target cells. Also comprehended are polypeptide fragments duplicating only a part of the continuous amino acid sequence or secondary conformations within OB receptor, which 25 fragments may possess one activity of mammalian OB receptor (e.g., immunological activity) and not others (e.g., OB protein binding activity).

Of applicability to OB receptor fragments and polypeptide analogs of the invention are reports of the 30 immunological activity of synthetic peptides which substantially duplicate the amino acid sequence extant in naturally-occurring proteins, glycoproteins and nucleoproteins. More specifically, relatively low molecular weight polypeptides have been shown to participate in immune 35 reactions which are similar in duration and extent to the

immune reactions of physiologically significant proteins such as viral antigens, polypeptide hormones, and the like.

Included among the immune reactions of such polypeptides is the provocation of the formation of specific antibodies in immunologically active animals. See, e.g., Lerner et al.,

- Cell 23: 309-310 (1981); Ross et al., Nature 294: 654-656 (1981); Walter et al., PNAS-USA 77: 5197-5200 (1980); Lerner et al., PNAS-USA, 78: 3403-3407 (1981); Walter et al., PNAS-USA 78: 4882-4886 (1981); Wong et al., PNAS-USA 79: 5322-5326 (1982); Baron et al., Cell 28: 395-404 (1982); Dressman et al., Nature 295: 185-160 (1982); and Lerner, Scientific American 248: 66-74 (1983). See, also, Kaiser et al. Science 223: 249-255 (1984) relating to biological and immunological activities of synthetic peptides which approximately share secondary structures of peptide hormones but may not share their primary structural conformation.

Thus, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

20. (a) 1-896;  
(b) 22-896;  
(c) 23-896;  
(d) 29-896  
(e) 1-839;  
25 (f) 22-839;  
(g) 29-839;  
(h) 1-841;  
(i) 22-841;  
(j) 23-841;  
30 (k) 29-841;  
(l) 1-891;  
(m) 22-981;  
(n) 23-891;  
(o) 29-891;

- (p) the amino acids of subparts (1) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);  
5 (q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

In addition, since the C-terminus region of the above polypeptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3 and 5) one may desire to prepare only the 10 polypeptides which are divergent:

- (a) those having only amino acids 892-896 of Seq. ID No. 1;  
(b) those having only amino acids 892-933 of Seq. ID No. 3;  
(c) those having only amino acids 892-959 of Seq. ID No. 5.

One may modify the OB receptor to create a fusion 15 molecule with other peptide sequence. For example, if one desired to "tag" the OB receptor with an immunogenic peptide, one could construct a DNA which would result in such fusion protein. The tag may be at the N-terminus. Alternatively, since it is apparent that the C-terminus is not necessary for 20 ligand binding activity, one may chemically modify the C-terminus. One may desire, for example, a preparation whereby one or more polymer molecules such as polyethylene glycol molecules are attached. Thus, another aspect of the present invention is chemically modified OB receptor protein.

25 The present invention also includes that class of polypeptides coded for by portions of the DNA complementary to the protein-coding strand of the human cDNA or genomic DNA sequences of OB receptor i.e., "complementary inverted proteins" as described by Tramontano et al. Nucleic Acid Res. 30 12: 5049-5059 (1984). Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

One may prepare soluble receptor by elimination of the transmembrane and intracellular regions.

Also comprehended by the invention are pharmaceutical compositions comprising effective amounts of polypeptide products of the invention together with pharmaceutically acceptable diluents, preservatives, 5 solubilizers, emulsifiers, adjuvants and/or carriers useful in OB receptor therapy. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, 10 Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); covalent attachment of polymers such as polyethylene glycol to the protein (as discussed *supra*, *see*, for example U.S. 15 patent 4,179,337 hereby incorporated by reference); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Such compositions will influence the physical state, stability, rate of *in vivo* 20 release, and rate of *in vivo* clearance of OB receptor. *See*, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference.

Generally, an effective amount of the present OB 25 receptor polypeptides will be determined by the age, weight and condition or severity of disease of the recipient. *See*, Remingtons Pharmaceutical Sciences, *supra*, at pages 697-773, herein incorporated by reference. Typically, a dosage of between about 0.001mg/kg body weight/day to about 1g/kg body 30 weight/day, may be used, but more or less, as a skilled practitioner will recognize, may be used. For local (i.e., non-systemic) applications, such as topical applications, the dosing may be between about 0.001g/cm<sup>2</sup> to about 1g/cm<sup>2</sup>. Dosing may be one or more times daily, or less frequently, 35 and may be in conjunction with other compositions as

described herein. It should be noted that the present invention is not limited to the dosages recited herein.

Polypeptide products of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with  $^{125}\text{I}$ ) to provide reagents useful in detection and quantification of OB receptor in solid tissue and fluid samples such as blood or urine. Nucleic acid products of the invention may also be labeled with detectable markers (such as radiolabels and non-isotopic labels such as 5 biotin) and employed in hybridization processes to locate the 10 human OB receptor gene position and/or the position of any related gene family in a chromosomal map. Nucleic acid sequences which selectively bind the human OB receptor gene 15 are useful for this purpose. They may also be used for identifying human OB receptor gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders. Contemplated herein are kits containing such labelled materials.

The nucleic acids provided herein may also be 20 embodied as part of a kit or article of manufacture. Contemplated is an article of manufacture comprising a packaging material and one or more preparations of the presently provided nucleic acids. Such packaging material will comprise a label indicating that the nucleic acid 25 preparation is useful for detecting OB receptor or OB receptor defects in a biological sample. As such, the kit may optionally include materials to carry out such testing, such as reagents useful for performing DNA or RNA hybridization analysis, or PCR analysis on blood, urine, or 30 tissue samples.

A further embodiment of the invention is selective binding molecules, such as monoclonal antibodies selectively binding OB receptor. The hybridoma technique described originally by Kohler and Milstein Eur. J. Immunol. 6, 511-519 35 (1976) has been widely applied to produce hybrid cell lines

that secrete high levels of monoclonal antibodies against many specific antigens. Recombinant antibodies, (see Huse et al., Science 246: 1275 (1989)) may also be prepared. Such recombinant antibodies may be further modified, such as by modification of complementarity determining regions to increase or alter affinity, or "humanizing" such antibodies. Such antibodies may be incorporated into a kit for diagnostic purposes, for example. A diagnostic kit may be employed to determine the location and/or amount of OB receptor of an individual. Diagnostic kits may also be used to determine if an individual has receptors which bind OB protein, or those which, to varying degrees, have reduced binding capacity or ability. As stated *infra*, such antibodies may be prepared using immunogenic portions of an OB receptor protein. Such selective binding molecules may themselves be alternatives to OB protein, and may be formulated for pharmaceutical composition.

RELATED METHODS

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The present compositions may be used in therapeutic as well as diagnostic methods.

25

The present OB receptor proteins or nucleic acids may be used for methods of treatment, or for methods of manufacturing medicaments for treatment. Such treatment includes conditions characterized by excessive production of OB protein, wherein the present OB receptors, particularly in soluble form, may be used to complex to and therefore inactivate such excessive OB protein. This treatment may be accomplished by preparing soluble receptor (e.g., use of the extracellular domain) or by preparation of a population of cells containing such OB receptor, and transplanting such cells into the individual in need thereof. The present OB receptors may also be used for treatment of those having defective OB receptors. For example, one may treat an

individual having defective OB receptors by preparation of a population of cells containing such non-defective OB receptor, and transplanting such cells into an individual. Or, an individual may have an inadequate number of OB receptors, and 5 cells containing such receptors may be transplanted in order to increase the number of OB receptors available to an individual. Such treatment may be for the purpose of modulating weight loss, for therapeutic purposes or solely for cosmetic purposes.

10 The present OB receptor protein or nucleic acids may be used for diagnostic purposes. For instance, RNAs or DNAs may be used to characterize or detect defects in an individual's OB receptors. For example, an obese individual may possess OB receptors which are characterized by a reduced 15 ability to bind OB protein. The present DNAs may be used to hybridize with the nucleic acid from an individual to detect such defects, such as via PCR techniques. OB receptor protein may be used to characterize an individual's OB protein for its ability to bind to OB receptor, or for other biological 20 activity. For example, one may prepare an assay for the ability of OB protein to alter lipid metabolism by preparing a population of lipid containing cells expressing the OB receptor, and contacting OB protein with such population of cells. Modulation of lipid content, characteristics of lipid 25 or other characteristics may be monitored. For diagnostic purposes, the present protein or nucleic acids may be associated with a detectable labels substance such as a radioactive isotope, a fluorescent or chemiluminescent chemical, or other label available to one skilled in the art. 30 Such nucleic acids may be used for tissue distribution assays (for example, as provided in the working example below) or for other assays to determine the location of OB receptor.

The present OB receptor protein family may be used in methods to obtain OB protein analogs, mimetics or small 35 molecules. One would simply prepare a desired OB receptor

protein, particularly one with capability of binding to native OB protein, and assay the test molecule, which may be labelled with a detectable label substance, for ability to bind to such receptor. Other parameters, such as affinity, and location of binding, may also be ascertained by methods available to those skilled in the art. For example, one could use portions of the present OB receptors, particularly portions in the extracellular domain which are necessary for ligand binding, to determine the location of such binding.

5 One could prepare OB receptors which have various truncations or deletions of regions of the extracellular domain which could be used to determine the location of test molecule binding. One could use an OB receptor known to be defective in native OB binding, such as potentially one from an individual having such defective receptors, and use this as the basis for ascertaining OB protein which would be effective to result in desired biological activity (i.e., weight loss, reduction in blood dyslipidemias or lowering of cholesterol levels, reductin in incidence of diabetes). Other 10 uses include solely cosmetic uses for alteration of body appearance, particularly the removal of fat.

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The present OB receptor protein or nucleic ac. is may also be useful to identify substances which "up-regulate" OB protein or receptor. For instance, the temporal expression of OB receptor in vivo may abe useful to determine if an administered substance causes an increase or decrease in OB receptor. One may conclude that an increase in OB receptor expression results in modultion of weight or lipid metabolism.

25

30 The divergence in the C-terminus may represent OB receptors with different signal transduction abilities. Therefore the different receptor family members may be used for differing assays, depending on the type of signal transduction observed.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

5 **EXAMPLE 1: IDENTIFICATION OF HUMAN OB RECEPTOR PROTEIN**

Human OB receptor protein DNA was identified in a human liver cDNA library in two steps. The first step used two primers in polymerase chain reaction (PCR) primers to 10 amplify a selected 300 base pair region from the human liver cDNA library. The second step used the PCR fragment as a probe to screen the human liver cDNA library. Thirteen clones were obtained, but these were incomplete at the 5' end. A procedure was performed to complete the 5' end to 15 make complete clones. Twelve clones were sequenced. These twelve clones were identified as either "A", "B" or "C" as denoted by the C'terminus of the predicted amino acid sequence.

20 **Polymerase Chain Reaction.** The original PCR primer was based on the 5' end and the 3' end of a 416 base pair sequence having GenBank Database Accession No. T73849. This sequence was selected on the basis of a known motif present in cytokine receptors, "WSXWS".

25 The 5' primer had the sequence 73-96 of the 416 bp sequence. The 3' primer had the sequence 337-360 of the 416 bp sequence.

These primers were used to probe a human cDNA liver library (Stratagene). Standard methods were used.

30 This resulted in a PCR fragment having the sequence 73-360 of the 416 bp fragment.

**Hybridization.** The 300 bp PCR fragment was used to probe a human liver cDNA library (Stratagene) using standard

methods. This second hybridization resulted in 13 positive clones. These were partial clones, incomplete at the 5' end.

Completion of the 5' end. Rapid Amplification of cDNA End ("RACE", kit, GIBCO/BRL) was used to obtain the full length clones.

Sequencing results. Sequencing revealed the three types of OB receptor DNAs. Of the thirteen clones, 4 clones were the "A" type (Seq. ID Nos. 1 and 2); 1 clone was the "B" type (Seq. ID Nos. 3 and 4) and 4 clones were of the "C" type (Seq. ID Nos. 5 and 6).

As can be seen from the Sequence Identifications (below), OB receptor A is 896 amino acids long, "B" is 933 amino acids long, and "C" is 959 amino acids long. These different OB receptors are identical at amino acid positions 1-891, and diverge almost completely beginning at position 892. The leader sequence is postulated to be, by hydrophobicity analysis, amino acids 1-21(M-A), 1-22(M-F) or 1-28(M-I), with the mature protein beginning at positions 22(F), 23(N) or 29(T). Based on hydrophobicity analysis, the leader sequence is most likely to be at positions 1-21(M through A). The transmembrane region is likely to begin at either position 840 (A) or 842(L) through position 862(I). 863(S) or 864(H). For OB receptor type A, the last amino acid is located at position 896 and is a lysine (L).

EXAMPLE 2: TISSUE DISTRIBUTION

Tissue distribution was ascertained using two methods. The first method involved using the entire type "A" OB receptor. The second method involved using probes which are specific to the C-terminal region of the protein. Since these C terminal regions are divergent, the second method

detected the tissue distribution of the different members of the OB receptor family.

The first method used a Northern Blot kit (Clontech), using the entire type A OB receptor DNA as a probe. The second method used PCR with primers specific to the nucleic acids encoding the divergent C terminus of the three types. Standard methods were used.

Table 1 shows the results for the Northern Blot and the PCR methods. The "+" indicates the investigator's subjective determination of the strength of signal. For the Northern Blot analysis, a triple "+++" indicates that a result (a dark "band" on the X-ray film) was seen upon overnight exposure of the film. A double "++" indicates that bands were seen at two weeks of exposure. A single "+" indicates that the bands were seen after three weeks of exposure. In addition, using this method, two molecular weights were observed, one at 4 Kb and one at 6.2 Kb. Although distribution was ubiquitous, the strongest signals were seen for ovary, heart and liver. For the PCR analysis, OB receptor "A" was seen in all tissue types tested (prostate, ovary, small intestine, heart, lung, liver and skeletal muscle), type "B" was seen only in lung and liver, and type "C" was seen in ovary, heart, lung and liver.

Table 1

Tissue Distribution of the Novel OB Receptor

5

	Northern Blot		PCR		
	4 Kb	6.2 Kb	A	B	C
Spleen	-	+			
Thymus	-	+			
Prostate	-	+	+	-	-
Testis	-	+			
Ovary	-	+++	+	-	+
Small Intestine	-	++	+	-	-
Colon	-	-			
Peripheral blood	-	-			
Leukocyte					
Heart	-	+++	+	-	+
Brain	-	-			
Placenta	-	+			
Lung	+	++	+	+	+
Liver	+++	+++	+	+	+
Skeletal Muscle	-	++	+	-	-
Kidney	-	++			
Pancreas	-	+			

10 EXAMPLE 3: IDENTIFICATION OF HUMAN OB RECEPTOR GENOMIC DNA AND CHROMOSOME LOCALIZATION

15 The full-length human OB receptor genomic DNA was also prepared. OB receptor "A" cDNA, in its entirety, was used as a probe against a human genomic DNA library, using materials and methods from a commercially available kit (Genome Systems, using a human genomic library in a P1 vector). A single positive clone was detected. There are 20 introns located at (with respect to OB receptor "A" DNA) base pair number: 559, 1059, 1350, 1667, 1817, 1937, 2060, 2277, 2460, 2662, and 2738.

The human OB receptor gene was localized to human chromosome 1P31 by FISH analysis (Genome Systems). Human chromosome 1 is thought to correspond to mouse chromosome 4C7, which is presumed to be the location of the db locus.

5

EXAMPLE 4: PREPARATION OF EXPRESSION VECTORS

Recombinant human OB receptor expression vectors have been prepared for expression in mammalian cells. As indicated above, expression may also be in non-mammalian cells, such as bacterial cells. The type "A" cDNA (Seq. ID No. 2) was placed into a commercially available mammalian vector (CEP4, Invitrogen) for expression in mammalian cells, including the commercially available human embryonic kidney cell line, "293". For expression in bacterial cells, one would typically eliminate that portion encoding the leader sequence (e.g., potentially amino acids 1-21, 1-22 or 1-28). One may add an additional methionyl at the N-terminus for bacterial expression. Additionally, one may substitute the native leader sequence with a different leader sequence, or other sequence for cleavage for ease of expression.

EXAMPLE 5: PREPARATION OF SELECTIVE BINDING MOLECULES

Animals were immunized for the preparation of polyclonal antibodies using the following peptides (with respect to the numbering of the amino acids for OB receptor A, Seq. ID No. 1): 54-64; 91-100; 310-325; 397-406; 482-496; 874-885; and, with respect to amino acids of OB receptor 30 "C" (Seq. ID No. 5), 910-929.

Other immunogenic peptides may be used.

Polyclonal, monospecific polyclonal, monoclonal, antibody fragments, and recombinant antibodies may be prepared using methods available to those skilled in the art.

One may further use recombinant techniques or peptide synthesis methods to alter the character of the such selective binding molecules. This may be accomplished by preparing recombinant antibodies having altered complementarity determining regions (sometimes referred to in the art as "CDR's") to, for example "humanize" the antibodies by using human Fc (constant) regions. Other types of recombinant antibodies, for example, those having CDR's altered to enhance affinity or selectivity to one or more members of the OB receptor family, may be prepared and used using methods available to those skilled in the art. See Winter et al., Nature 349: 293-299 (1991).

The present OB receptor protein may be used as an assay to screen for desired selective binding molecules. Such assay may be based on binding capability, or biological activity, or, other means of detecting signal transduction. For example, if one were to prepare a series of modified antibodies, one could test them for affinity (i.e., binding strength) against the target OB receptor.

The selective binding molecules may be useful for diagnostic purposes, such as tissue distribution analysis, or to diagnose the relative affinity of an individual's OB receptors for such selective binding molecule to determine the functionality of an individual's OB receptor during a course of therapy. Selective binding molecules may be alternative therapeutic or cosmetic products to OB protein.

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

Human OB Receptor "A" Amino Acid Sequence (Seq. ID No.  
11 Amino Acid, single letter abbreviation. "\*" indicating stop  
codon:

5        1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP N STYDYFLLP  
      51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC F RSEQDRNCS  
10      101   LCADNIEGKT FVSTVNSLVE QQIDANWNIQ CWLKGDLKLF I CYVESLFKN  
      151   LFRNYYNYKVN LLYVLPEVLE DSPLVPQKGS FQMVHCNCV HECCECLVPV  
      201   PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI L GLHMEITDD  
15      251   GNLKISWSSP PLVPPFLQYQ VKYSENSTV IREADKIVSA TSLLVDSILP  
      301   GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI L TSVGSNVSF  
20      351   HCYKKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVS K VTFFNLNETK  
      401   PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG Y LTKMTCRWS  
      451   TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL Q SDGFYECIF  
25      501   QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP S SVKAETIN  
      551   IGLLKISWEK PVFPENNLLQF QIRYGLSGKE VQWKMYEVYD A KSKSVSLPV  
30      601   PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
      651   GDTMKKEKNV TLLWKPLMKN DSLCSVQRV INHHTSCNGT WSEDVGHNHTK  
      701   FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI V QSL SAYPLN  
35      751   SSCVIVSWIL SP SDYKLMYF I LEWKLNED GEIKWLRSS SVKKYYIHDM  
      801   FIPIEKYQFS L YPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
40      851   SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ K RTDIL\*SLI  
      901   MITTDEPNVP TSQQSIEY\*K IFTF\*RRGAN LKKIQLNF\*E L TYGGLC\*FR  
45      951   T\*NRCVNLGS KCRFESSIONLDV \*L

Human OB Receptor "A" DNA Sequence (Seq. ID No. 2 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTACA  
10 101 TTGGGAATT ATTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATT  
15 151 CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATT AACCTATGAC  
20 201 TACTTCCTT TGCCCTGCTGG ACTCTCAAAG AATACTCAA ATTCAATGG  
25 251 ACATTATGAG ACAGCTGTTG AACCTAAGTT TAAITCAAGT GGTACTCACT  
30 301 TTTCTAACCT ATCCAAAACA ACTTTCCACT GTTGCTTCG GAGTGAGCAA  
35 351 GATAGAAAAC GCTCCTTATG TGCAAGACAAC ATTGAAGGAA AGACATTGT  
40 401 TTCAACAGTA AATTCTTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCACTG TTATGTGGAG  
50 501 TCATTATTAA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATT ACCTCTGGTT CCCCCAAAAG  
60 601 GCAGTTTCA GATGGTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA  
65 651 TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTT CCAGTCACCT CTAATGTCAG  
75 751 TTCAGGCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAGATT TCTTGGTCCA GCCCACATT  
85 851 GGTACCATT CCACCTCAAT ATCAAGTGAA ATATTCAAGAG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAAC TTCCCTGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA  
105 1051 CCACACAAAGA TGTCAATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAATGTTT CTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTAAA  
120 1201 GCCAGTATGA TGGTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT

1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG  
1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATT AATGTGATTG  
5 1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCAG AAAGCACTTT  
1451 GCAATTGAGG TATCATAGGA GCAGCCTTA CTGTTCTGAT ATTCCATCTA  
10 1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTT  
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAAATGTG  
15 1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAAACATGTG  
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT  
20 1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGAAA ATCAAATCT  
25 1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG  
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
1951 CCTACACAGT TGTCAATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
30 2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACCTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
35 2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTGCA AATTTTAATT  
40 2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCCCTGGA TACTATCACC  
45 2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT  
50 2451 TATATCCATG ATCATTATTT CCCCCATTGAG AAGTACCAAGT TCAGTCTTTA  
2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA  
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGAA CATTATTAAT  
15 2651 ATCACACCAA AGAATGAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
2701 AGAATTGTTCTGGGCACAA GGACTTAATT TTCAGAAGAG AACGGACATT  
2751 CTTTGAAGTC TAATCATGAT CACTACAGAT GAACCCAATG TGCCAACTTC  
10 2801 CCAACAGTCT ATAGAGTATT AGAAGATTT TACATTTGA AGAAGGGGAG  
2851 CAAATCTAAA AAAAATTCAAG TTGAACCTCT GAGAGTTAAC ATATGGTGGA  
2901 TTATGTTGAT TTAGAACTTA AAATAGATGT GTAAATTGG GTTCAAAATG  
15 2951 TAGATTGAG TCCAGTTGG ATGTGTGATT AATTTCAAA TCATCTAAAG  
3001 TTTAAAAGTA GTATTCACTA TTTCTGGCTT TTGATTGCGC ATATTCCCTGG  
20 3051 TCATAAAACA TTAAGAAAAT TATGGCTGTT GCTGTCATTA CATATCTATT  
3101 AAATGTCATC AAATATGTAG TAGACAATTT TGTAATTAGG TGAACCTAA  
3151 AACTGCAACA TCTGACAAAT TGCTTTAAAA ATACAATGAT TAT

Human OB Receptor "B" Amino Acid Sequence (Seq. ID No. 3  
(Amino Acid):

5    1    MICQKFCVVL LHWEFIYVIT AFNLSSYPITP WRFKLSCMPP NSTYDYFLLP  
      51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
    101    LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
    151    LFRNYYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCV HECCECLVPV  
    201    PTAKLNDTLL MCLKITSGGV IFQSPPLMSVQ PINMVKPDPPI LGLHMEITDD  
    251    GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
    301    GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSE  
    351    HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVK VTFFNLNETK  
    401    PRGKFETYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS  
    451    TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
    501    QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAETIN  
    551    IGLLKISWEK PVFPENNLFQ QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
    601    PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
    651    GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGHNHTK  
    701    FTFLWTEQAH TVTVLAINSII GASVANFNLT FSWFMSKVNI VQSLISAYPLN  
    751    SSCVIVSWIL SPSDYKLMYF IIIEWKNLNED GEIKWLRSS SVKKYYIHDH  
    801    FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
    851    SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KKRLSIFLSS  
    901    IQHQ\*HVVLF FWSLKQFQKI SVLIHHGKIK MR\*CQQLWSL YFQQQILKRV  
    951    LFVLVTSSTV LTSLRLRVLR \*PMRTKARDN PLLNTPR\*SA TLNQVKLVK

Human OB Receptor "B" DNA Sequence (Seq. ID No. 4 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTGGAGTTG GACCCCCGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTCACA  
10 101 TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATT  
15 151 CTCCTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC  
20 201 TACTTCCTTT TGCGCTGTGG ACTCTCAAAG AATACTTCAA ATTCAATGG  
25 251 ACATTATGAG ACAGCTGTGG AACCTAAGTT TAATTCAAGT GGTACTCACT  
30 301 TTTCTAACTT ATCCAAAACA ACTTCCACT GTTGCTTTCG GAGTGAGCAA  
35 351 GATAGAAAATC GCTCCTTATG TGCAAGACAAC ATTGAAGGAA AGACATTTGT  
40 401 TTCAACAGTA AATTCTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAT TATTCACTG TTATGTGGAG  
50 501 TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATT ACCTCTGGTT CCCCAAAAAG  
60 601 GCAGTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA  
65 651 TGTCTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTT CCAGTCACCT CTAATGTCAG  
75 751 TTCAGCCCCAT AAATATGGTG AAGCCTGATC CACCATAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCAATT  
85 851 GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAAGAG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAC TTCTGGTGC TTCTATGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA  
105 1051 CCACACAAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAAATGTT CTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTCAA  
120 1201 GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT

1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG  
1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTATATGTGATTG  
5 1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACCTGCGG AAAGCACTTT  
1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA  
10 1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT  
1551 TATGAATGCA TTTTCAGCC AATCTCCTA TTATCTGGCT ACACAATGTG  
15 1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG  
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT  
20 1751 CTTTCCAGAG AATAACCTTC AATTCCAG TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT  
25 1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAAGGTGCG  
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
1951 CCTACACAGT TGTCAATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
30 2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
35 2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTGCA AATTTTAATT  
40 2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCTGTGGAA TACTATCACC  
45 2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT  
2451 TATATCCATG ATCATTATTT CCCCATTGAG AAGTACCAAGT TCAGTCTTTA  
50 2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA  
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT  
2651 ATCACACCAA AGAATGAAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
5 2701 AGAATTGTTCTGGGCACAA GGACTTAATT TTCAGAAGAA ACGTTGAGC  
2751 ATCTTTTAT CAAGCATACA GCATCAGTGA CATGTGGTCC TCTTCTTTG  
10 2801 GAGCCTGAAA CAATTCAGA AGATATCAGT GTTGATACAT CATGGAAAAA  
2851 TAAAGATGAG ATGATGCCAA CAACTGTGGT CTCTCTACTT TCAACAAACAG  
2901 ATCTTGAAGGGGGTTCTGTT TGTTTAGTG ACCAGTTCAA CAGTGTAAAC  
15 2951 TTCTCTGAGG CTGAGGGTAC TGAGGTAACC TATGAGGACG AAAGCCAGAG  
3001 ACAACCCCTT GTAAATACCG CCACGCTGAT CAGCAACTCT AAACCAAGTG  
20 3051 AAACTGGTGA AGA

Human OB Receptor "C" Amino Acid Sequence (Seq. ID No. 5  
(Amino Acid):

5    1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP  
51    AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS  
101    LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN  
10    151    LFRNYNYKVKH LLYVLPEVLE DSPLVPQKGS FQMVHCNCV HECCECLVPV  
201    PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPPI LGLHMEITDD  
15    251    GNLKISWSSP PLVPFFLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP  
301    GSSYEVQVRG KRLDGPGIWS DWSTPRVFET QDVIYFPPPQI LTSVGSNVSF  
20    351    HCYIKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTEFFNLNETK  
401    PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTAKMTCRWS  
451    TSTIQSLAES TLQLRYHRSS LYCSDIPSIIH PISEPKDCYL QSDGFYECIF  
25    501    QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN  
551    IGLLKISWEK PVFPENNLFQ QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV  
601    PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN  
30    651    GDTMKKEKNV TLLWKPLMKN DSLCSVQRVYV INHHHTSCNGT WSEDVGNHTK  
701    FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSL SAYPLN  
35    751    SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRSS SVKKYYIHDK  
801    FIPIEKYQES LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII  
40    851    SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KMLEGSMFVK  
901    SHHHS LISST QGHKKHCGRPQ GPLHRKTRDL CSLVYLLTLP PLLSYDPAKS  
951    PSVRNTQE\*S IKKKKKLEG

Human OB Receptor "C" DNA Sequence (Seq. ID No. 6 (DNA)):

1 CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA  
5 51 CTTCTCTGAA GTAAGATGAT TTGTCAAAA TTCTGTGTGG TTTTGTACA  
10 101 TTGGGAATT ATTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA  
15 151 CTCCTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTG AACCTATGAC  
20 201 TACTTCCTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCAATGG  
25 251 ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT  
30 301 TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTCG GAGTGAGCAA  
35 351 GATAGAAAAC GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT  
40 401 TTCAACAGTA AATTCTTAG TTTTCAACA AATAGATGCA AACTGGAACA  
45 451 TACAGTGCTG GCTAAAAGGA GACTTAAAT TATTCACTG TTATGTGGAG  
50 501 TCATTATTTA AGAACCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT  
55 551 ATATGTTCTG CCTGAAGTGT TAGAAGATT ACCTCTGGTT CCCCCAAAAG  
60 601 GCAGTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA  
65 651 TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG  
70 701 TTTGAAAATC ACATCTGGTG GAGTAATTT CCAGTCACCT CTAATGTCAG  
75 751 TTCAGCCCAC AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG  
80 801 GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT  
85 851 GGTACCATT CCACCTCAAT ATCAAGTGAA ATATTCAAGG AATTCTACAA  
90 901 CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA  
95 951 GACAGTATAC TTCCCTGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG  
100 1001 ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA  
105 1051 CCACACAAAGA TGTCAATATAC TTTCCACCTA AAATTCTGAC AAGTGTGGG  
110 1101 TCTAATGTTT CTTCCTACTG CATCTATAAG AAGGAAAACA AGATTGTTCC  
115 1151 CTCAAAAGAG ATTGTTGGT GGATGAATT AGCTGAGAAA ATTCCCTAAA  
120 1201 GCCAGTATGA TGTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTCAAT

1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG  
1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATT AATGTGATTG  
5 1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG  
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT  
10 1451 GCAATTGAGG TATCATAGGA GCAGCCTTA CTGTTCTGAT ATTCCATCTA  
1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTT  
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG  
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG  
1651 TCCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA  
20 1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT  
1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA  
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT  
25 1851 GTCACTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG  
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG  
30 1951 CCTACACAGT TGTCAATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT  
2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT  
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT  
35 2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG  
2151 GGAAATCACA CGAAATTACAC TTTCTGTGG ACAGAGCAAG CACATACTGT  
40 2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTGCA AATTTAATT  
2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT  
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTCCTGGAA TACTATCACC  
45 2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG  
2401 AAGATGGTGA AATAAAATGG CTTAGAAATCT CTTCATCTGT TAAGAAGTAT  
50 2451 TATATCCATG ATCATTTTAT CCCCCATTGAG AAGTACCAAGT TCAGTCTTA  
2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTCA

2551 CTCAGATGA TATTGAAAAA CACCAAGAGTG ATCCAGGTTT ATATGTAATT  
2601 GTGCCAGTAA TTATTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT  
5 2651 ATCACACCAA AGAATGAAAA AGCTATTTG GGAAGATGTT CCGAACCCCA  
2701 AGAATTGTTCTGGGCACAA GGACTTAATT TTCAGAAGAT GCTTGAAGGC  
2751 AGCATGTTCG TTAAGAGTCA TCACCACTCC CTAATCTCAA GTACCCAGGG  
10 2801 ACACAAACAC TGCGGAAGGC CACAGGGTCC TCTGCATAGG AAAACCAGAG  
2851 ACCTTTGTTCACTTGTTTAT CTGCTGACCC TCCCTCCACT ATTGTCTTAT  
15 2901 GACCTTGCCA AATCCCCCTC TGTGAGAAC ACCCAAGAAT GATCAATAAA  
2951 AAAAAAAAAA AAAAAACTCG AGGGGG

CLAIMS

1. An OB receptor protein having part or all of the amino acid sequence according to Seq. ID No. 1.

2. An OB receptor protein selected from among 5 those having amino acids (according to Seq. ID No. 1):

- (a) 1-896;
- (b) 22-896;
- (c) 23-896;
- (d) 29-896
- 10 (e) 1-839;
- (f) 22-839;
- (g) 29-839;
- (h) 1-841;
- (i) 22-841;
- 15 (j) 23-841;
- (k) 29-841;
- (l) 1-891;
- (m) 22-981;
- (n) 23-891;
- 20 (o) 29-891;

(p) of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID.

No. 5); and

25 (q) of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

3. A DNA molecule encoding an OB receptor protein of claim 1 or 2.

30 4. A DNA molecule encoding an OB receptor protein selected from the group consisting of:

- (a) that of Seq. ID. Nos. 2, 4, or 6;
- (b) a DNA which hybridizes to a DNA of subpart (a)

(c) a DNA which, but for the degeneracy of the genetic code would hybridize to a DNA of subpart (a) or (b).

5. A biologically functional viral or plasmid vector containing a DNA of claim 3.

6. A biologically functional viral or plasmid vector containing a DNA of claim 4.

10 7. A prokaryotic or eukaryotic host cell containing the vector of claim 5.

8. A prokaryotic or eukaryotic host cell containing the vector of claim 6.

15 9. A host cell modified so that expression of endogenous OB receptor DNA is enhanced.

10. A host cell of claim 9 which is an isolated human  
20 host cell.

11. A process for producing an OB receptor comprised of culturing, under suitable conditions, a host cell of claim 7 or 8, and obtaining the OB receptor produced.

25 12. A selective binding molecule which selectively binds an OB receptor.

13. A selective binding molecule of claim 12 which  
30 selectively binds the extracellular domain of an OB receptor.

14. An OB receptor polypeptide selected from among:  
35 (a) those having only amino acids 892-896 of Seq. ID No. 1;  
(b) those having only amino acids 892-933 of Seq. ID No. 3;  
(c) those having only amino acids 892-959 of Seq. ID No. 5.

15. A DNA encoding the OB receptor polypeptide of claim  
14.
- 5 16. A biologically functional plasmid or viral vector  
containing the DNA of claim 15.
17. A prokaryotic or eukaryotic host cell containing  
the vector of claim 16.
- 10 18. A process for producing an OB receptor polypeptide  
comprised of culturing, under suitable conditions, the host  
cell of claim 17, and obtaining the OB receptor polypeptide  
produced.

P3

### DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

### OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

which is described and claimed in the specification which:

- is attached hereto.  
 was filed on JANUARY 4, 1996  
as Application Serial No.: 08/582825  
and was amended on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

3 Ron K. Levy, Registration No.: 31,539, Steven M. Odro, Registration No.: 29,094, and Karol M. Pessin, Registration No. 34,899, said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

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Date: Jan 21, 1996

### CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant  
Commissioner of Patents, Washington, D.C. 20231, on the date appearing below.

3/29/96

Umer Khan

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

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